Lab notebook: Cellulose polymerisation machinery for phase 2, 3 and considerations for Phase 4.

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Phase 2 & 3: Experimentally accessible manufacturing infrastructure

The V1 system is a minimal, experimentally tractable architecture for enzymatic cellulose synthesis inside a nanofluidic channel. Three functional subsystems are described below.

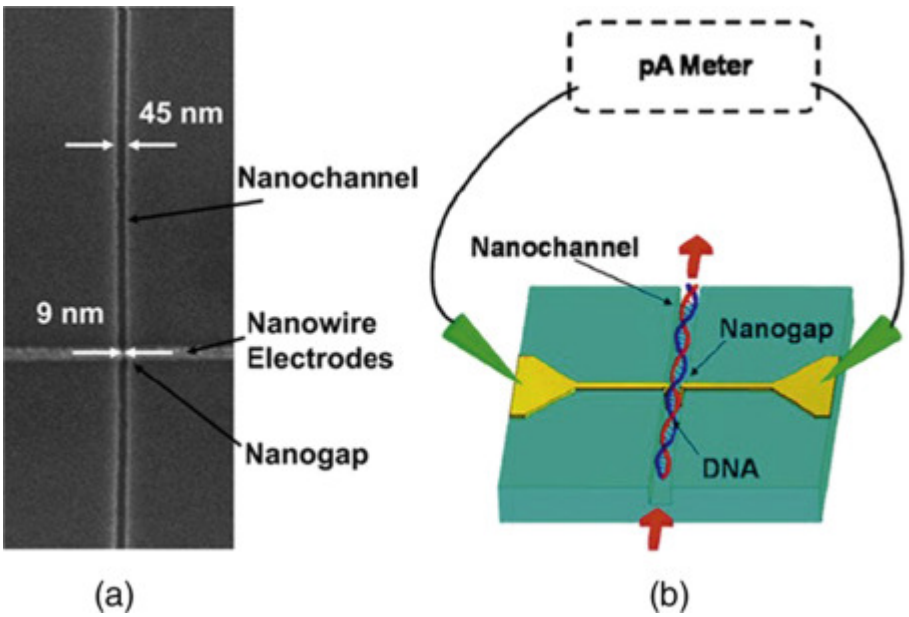
1. Glucose inlet
A single nanoscale inlet channel delivers glucose monomer feedstock into the reaction chamber.

2. Synthesis chamber
At the terminus of the inlet channel, a nanochamber houses immobilised/freefloating cellulose synthase enzymes. Upon glucose delivery, the enzymes catalyse β-1,4-glycosidic bond formation, producing linear cellulose chains. Chamber dimensions must accommodate enzyme turnover kinetics and product accumulation without steric occlusion.

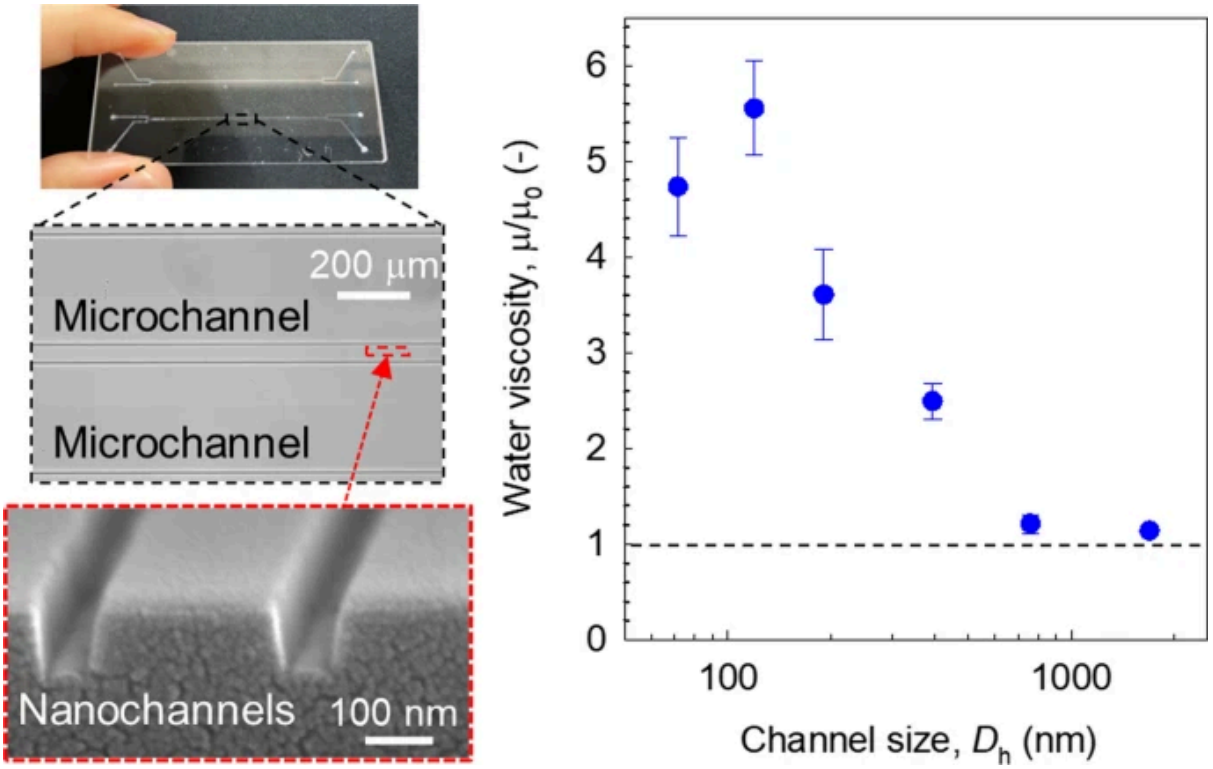
3. Enzyme-cellulose separation
Post-synthesis, the product stream must be separated from the enzyme population before extrusion or collection. Three candidate methods are under consideration and can be used in combination:

- **Electrophoretic/electroosmotic separation:** an applied electric field exploits charge differences between enzymes (amphoteric) and cellulose chains. Electroosmotic flow additionally leverages size-dependent mobility: smaller enzymes migrate faster than nascent cellulose strands and can be selectively swept out of the separation zone.
- **Dielectrophoresis:** a non-uniform AC field deflects the more polarisable cellulose relative to the less polarisable enzyme population, enabling label-free sorting without a net charge requirement.
- **Enzyme desorption via high-salt wash:** a brief flush with high-ionic-strength buffer disrupts non-covalent enzyme-cellulose binding (primarily hydrophobic and electrostatic contacts), releasing trapped enzyme from the product strand. The cellulose is then recovered by downstream collection or nanopore extrusion.

Examples of nanofluidic systems to draw inspiration from:



https://www.sciencedirect.com/topics/materials-science/nanofluidics



https://link.springer.com/article/10.1007/s44211-023-00311-x

Also, Oxford nanopore nanofluidic setups.

Phase 4, 5, + Advanced build layer: design considerations

Phase 4 extends the V1 framework with mechanical actuation and composite material deposition. The following capabilities are flagged for integration:

- **Lignin and adhesive co-deposition**: for geometries requiring composite mechanical properties, lignin and other structural dopants are introduced alongside cellulose. Co-deposition parameters (concentration, timing, spatial localisation) are geometry-dependent.
- **Piezo chain-direction tool:** a piezoelectric actuator applies tensile or directional force to nascent chains during synthesis, biasing their packing orientation. This is the primary mechanism for directing assembly into target 3D geometries.
- **Sliding plate for product advance:** a lateral sliding element displaces completed cellulose product away from the active synthesis zone, clearing the chamber for the next build cycle and enabling continuous or semi-continuous operation.